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ScienceDirect
Veterinary Microbiology 124 (2007) 375–381
veterinary microbiology
www.elsevier.com/locate/vetmic

Short communication

Comparison of the antibacterial activity of honey from different provenance against bacteria usually isolated from skin wounds

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Received 27 December 2006; received in revised form 18 April 2007; accepted 25 April 2007

Abstract

The antibacterial activity of honey samples provided by apiarists and honey packers was tested against microorganisms usually isolated from skin wounds. The antibacterial activity was tested using the well-agar diffusion assay. The honey samples were tested without dilution, and at 75, 50, 30, and 10% (w/v) dilution. Most of the undiluted honey samples inhibited the growth of *Staphylococcus aureus* and *Staphylococcus epidermidis*. Some honey samples provided by apiarists also inhibited the growth of *S. aureus* even at 50% dilution. Undiluted honey samples also inhibited the growth of *Staphylococcus uberis*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae*, although to a lesser extent. No inhibition of *Micrococcus luteus* and *Enterococcus faecalis* growth was detected. The diameters of the inhibition zones generated by honey samples provided by apiarists were larger than those generated by honey samples provided by honey packers. This observation may be explained by considering the provenance of the honey samples.

Keywords: Honey; Antibacterial activity; Honey source

1. Introduction

Antibiotic-resistant bacteria continue to be of major health concern world-wide. Since the use of antibiotics became widespread over 50 years ago, bacteria have progressively developed resistance (Hsueh et al., 2005). Consequently, scientific efforts have been made to study and develop new compounds to be used beyond conventional antibiotic therapy.

Honey has been used since ancient times for the treatment of some respiratory diseases and for the healing of skin wounds. It has been proposed that the healing effect of honey could be due to various physical and chemical properties (Russell et al., 1990; Snow and Manley-Harris, 2004). The high osmolarity and acidity of honey are among the physical

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0378-1135/$ – see front matter © 2007 Published by Elsevier B.V.
doi:10.1016/j.vetmic.2007.04.039

characteristics that contribute to its antibacterial activity. Hydrogen peroxide, volatiles, organic acids, flavonoids, beeswax, nectar, pollen and propolis are important chemical factors that provide antibacterial properties to honey. Honey also contains oligosaccharides in small quantities. In a recent work, Shin and Ustunol related the sugar composition of honeys from different floral sources to the growth inhibition of various intestinal bacteria (Shin and Ustunol, 2005). All these physical and chemical factors give honey unique properties as a wound dressing: it has a rapid clearance of infections, rapid debridement of wounds, rapid suppression of inflammation, minimisation of scarring, and stimulation of angiogenesis as well as tissue granulation and epithelium growth (Molan, 2002).

Honey, as most natural products, may have a large variance in therapeutic components depending on its origin. Thus, the floral source of honey plays an important role on its biological properties. For example, Manuka honey from New Zealand is recognised for its therapeutic properties (Molan, 2002). The composition of honey has been shown to depend largely on its floral source (White, 1979). In consequence, it would not be surprising that the provenance of honey could determine its antibacterial properties. It is also possible that the mixing of honeys affect their antibacterial activity since honeys with lower antibacterial activities may mask the higher antibacterial activity of other honeys.

On the other side, different bacteria are responsible for wound contamination, wound colonization, or clinical infection. Microorganisms such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Micrococcus luteus*, *Streptococcus uberis*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae* are frequently isolated from skin wounds in humans and animals. Methicillin-resistant and -sensitive *S. aureus* (MRSA and MSSA) are the main strains involved in difficult-to-treat skin and underlying tissue infections associated with gram-positive bacteria (Halcón and Milkus, 2004). *S. epidermidis* infections are commonly acquired in the hospital as a result of contamination of surgical cuts with microorganisms from the patients themselves or from the hospital personnel (Vuong and Otto, 2002). Infection with *P. aeruginosa* is the most serious complication in burns patients (Nasser et al., 2003; Altoparlak et al., 2005), followed by infections with *K. pneumoniae*, *E. coli*, *S. aureus* and other pathogen microorganisms (Nasser et al., 2003). As mentioned earlier, honey has long been known to possess antibacterial properties and has an established usage as wound dressing (Molan, 1999; Cooper et al., 2002), although not all honeys are equally effective for wound healing (Molan, 2002).

Argentine honeys are recognised throughout the world because of their quality. However, there is little scientific research published about the microbiological properties of Argentina's honeys. Thus, we carried out a study to determine the antibacterial activity of some honeys produced in southern Córdoba (Argentina), and to correlate that antibacterial activity with the honey provenance.

2. Methodology

2.1. Honey samples

Honey samples were obtained from two sources: 5 honey samples were provided by honey packers and 10 honey samples were provided by local apiarists. Honey samples provided by local apiarists were obtained by draining the honey after manually uncapping the comb frames. Honey samples provided by honey packers were directly obtained from the 330 kg barrels. At the packing plant honey is extracted by centrifugation after mechanical uncapping of the comb frames. Then, it is settled in a pool, filtered and finally stored in the barrels. These barrels contain honey from different sources. All the honey samples were transferred into sterile plastic flasks, and stored in a fresh (23–25 °C) and dark place. For the antibacterial tests honey samples were used undiluted and at 75, 50, 30 and 10% dilutions (grams of honey diluted to a final volume of 100 mL).

2.2. Bacterial strains

Strains of *S. aureus*, *S. epidermidis*, *M. luteus*, *S. uberis*, *E. faecalis*, *P. aeruginosa*, *E. coli*, and *K. pneumoniae* were kindly donated by the Microbiology Laboratory of Universidad Nacional de Río Cuarto. Bacterial cultures were kept in brain–heart agar broths with 15% glycerol and maintained in 3 mL plastic bottles at −20 °C. All the bacterial cultures were diluted

until an Optical Density (O.D.) in the range of 0.02–0.04 was obtained. This O.D. value was equivalent to a bacterial concentration between 10^6 and 10^8 cells/ml.

2.3. Assessment of antibacterial activity

An agar-well diffusion assay was used with plates cultured with each of the above-mentioned bacterial strains. The glass plates (24 cm wide, 20 cm long and 5 cm deep) were cleaned with acidic alcohol and sterilised with an UV lamp during 45 min.

One millilitre of the bacterial culture was added to 165 mL of the hot (45 °C) culture media. After homogenization, 150 mL were poured into the glass plates and kept at 4 °C for 1 h. The remaining 15 mL were poured into regular Petri dishes used as controls. Holes about 4 mm diameter were drilled on the culture media and 50 μL of each honey dilution were added to each column. Each glass plate contained five columns (one column per dilution) and four rows (four repetitions), so that the four holes in the first column contained honey at 100%, the four holes of the second column contained honey at 75%, and so on. The plates thus prepared were incubated at 37 °C for 24 h. The diameters of the circular inhibition zones obtained after that period of time were measured with a calliper.

The minimum inhibitory concentration (MIC) of each honey sample on *S. aureus* was estimated by the agar-well diffusion method (National Comittee for Clinical Laboratory Standards, 1999). The MIC was estimated as the minimum honey concentration which showed a measurable inhibition zone.

2.4. Statistical data analysis

Results are expressed as mean ± standard deviation. ANOVA tests were ran at a confidence level of 95% when comparing two means.

3. Results

3.1. Antibacterial activity of honey samples provided by apiarists

The results of the inhibition tests ran with honey samples provided by local apiarists on the bacterial strains used in this study are shown in Table 1. It was observed that *S. aureus* was the most inhibited bacterial strain; all honey samples inhibited its growth. The average diameter of the inhibition zones produced by the undiluted honeys samples was 17.1 ± 0.1 mm. Honey samples C and D showed antibacterial activity at 50% dilution, with an average diameter of the inhibition zone of 7.3 ± 0.1 mm. Sample D showed the highest antibacterial activity, showing inhibition zone diameters of approximately 22 mm when used undiluted and inhibition zone diameters of approximately 8 mm at 50% dilution. No inhibition of bacterial growth was observed when honey was diluted at 30 or 10%.

The growth of *S. epidermidis* was also inhibited by these honey samples. Six out of the 10 honey samples (60%) inhibited this bacteria's growth.

The growth of other bacterial species was also inhibited by these honey samples, although to a lesser extent. Thus, *S. uberis* was inhibited by 5 of the 10 honey samples (50%), *E. coli* by 4 (40%), *K. pneumoniae* by 2 (20%) and *P. aeruginosa* also by 2 out of 10 honey samples (20%). *M. luteus* and *E. faecalis* bacterial growth were not affected in a noticeable way by these honey samples.

3.2. Antibacterial activity of honey samples provided by honey packers

The results of the inhibition tests ran with honey samples provided by honey packers on the bacterial strains used in this study are shown in Table 2.

All the undiluted honey samples (except sample named L) produced inhibition of *S. aureus* growth. The average diameter of the inhibition zones produced by these honeys samples was 13.2 ± 0.1 mm. No inhibition of bacterial growth was observed when honey was diluted at 75, 50, 30 or 10%.

Honey samples provided by the honey packers also inhibited the growth of *S. epidermidis*. Three out of five honey samples (60%) inhibited the growth of this microorganism.

Finally, only the growth of *P. aeruginosa* was inhibited by one of the honey samples provided by the honey packers (sample named N).

3.3. Phenol coefficients

The phenol coefficient estimated for honey samples A, B, E, F, G, H, I, J, K, M, N, and P was 0.05. The

Table 1
Inhibition of different bacterial strains by honey samples provided by apiarists

Honey sample	Honey dilution	*S. aureus*	*S. epidermidis*	*M. luteus*	*S. uberis*	*E. faecalis*	*E. coli*	*K. pneumoniae*	*P. aeruginosa*
A	Undiluted	+	−	−	−	−	−	−	−
	75%	−	−	−	−	−	−	−	−
	50%	−	−	−	−	−	−	−	−
B	Undiluted	++	−	−	−	−	−	−	−
	75%	−	−	−	−	−	−	−	−
	50%	−	−	−	−	−	−	−	−
C	Undiluted	++	−	−	+	−	−	+	−
	75%	+	−	−	−	−	−	−	−
	50%	+	−	−	−	−	−	−	−
D	Undiluted	+	+	−	−	−	−	+	−
	75%	+	−	−	−	−	−	−	−
	50%	+	−	−	−	−	−	−	−
E	Undiluted	++	+	−	+	−	−	−	−
	75%	−	−	−	−	−	−	−	−
	50%	−	−	−	−	−	−	−	−
F	Undiluted	++	+	−	+	−	+	−	+
	75%	−	−	−	−	−	−	−	−
	50%	−	−	−	−	−	−	−	−
G	Undiluted	++	+	−	−	−	+		+
	75%	−	−	−	−	−	−	−	−
	50%	−	−	−	−	−	−	−	−
H	Undiluted	++	+	−	+	−	+	−	−
	75%	−	−	−	−	−	−	−	−
	50%	−	−	−	−	−	−	−	−
I	Undiluted	++	+	−	+	−	+	−	−
	75%	−	−	−	−	−	−	−	−
	50%	−	−	−	−	−	−	−	−
J	Undiluted	+	−	−	−	−	−	−	−
	75%	−	−	−	−	−	−	−	−
	50%	−	−	−	−	−	−	−	−

++ represents an inhibition zone average diameter in the range from 15 to 24 mm; + represents an inhibition zone average diameter in the range from 5 to 15 mm, and − represents no inhibition.

phenol coefficient estimated for honey samples C and D was 0.1.

4. Discussion and conclusions

The average diameter of the inhibition zones (17.1 ± 0.1 mm) produced by honey samples provided by the apiarists were slightly larger than those produced by honey samples provided by honey packers (13.2 ± 0.1 mm). Although the number of honey samples analysed in this work was not high enough to obtain significant differences, it may in principle be thought that the difference in average diameters of the inhibition zones might be due to the slight differences observed in the honey extraction and storing up processes used. Honeys obtained by apiarists were not heat-treated. Also, the honey obtained from each apiary was not mixed with honeys from other apiaries. On the contrary, honey samples provided by honey packers suffered some heat treatment during the comb uncapping process. Furthermore, and maybe more importantly, honeys coming from different apiaries were mixed in the

Table 2
Inhibition of different bacterial strains by honey samples provided by honey packers

Honey sample	Honey dilution	*S. aureus*	*S. epidermidis*	*M. luteus*	*S. uberis*	*E. faecalis*	*E. coli*	*K. pneumoniae*	*P. aeruginosa*
K	Undiluted	+	+	–	–	–	–	–	–
	75%	–	–	–	–	–	–	–	–
	50%	–	–	–	–	–	–	–	–
L	Undiluted	–	–	–	–	–	–	–	–
	75%	–	–	–	–	–	–	–	–
	50%	–	–	–	–	–	–	–	–
M	Undiluted	+	–	–	–	–	–	–	–
	75%	–	–	–	–	–	–	–	–
	50%	–	–	–	–	–	–	–	–
N	Undiluted	+	+	–	–	–	–	–	+
	75%	–	–	–	–	–	–	–	–
	50%	–	–	–	–	–	–	–	–
P	Undiluted	++	++	–	–	–	–	–	–
	75%	–	–	–	–	–	–	–	–
	50%	–	–	–	–	–	–	–	–

++ represents an inhibition zone average diameter in the range from 15 to 24 mm; + represents an inhibition zone average diameter in the range from 5 to 15 mm, and – represents no inhibition.

settling chamber before they were transferred to the 330 kg barrels. We speculate that these characteristics may be responsible for the difference observed in the honeys antibacterial activities.

As for the antibacterial activity of honeys on the different bacterial strains, it was observed that *S. aureus* was the most inhibited bacterial strain, with 14 honey samples out of 15 inhibiting its growth (about 93%, see Tables 1 and 2). However, all the samples (100%) provided by apiarists inhibited the growth of *S. aureus*, while only four out of five (80%) of the samples provided by honey packers inhibited the growth of *S. aureus*. Moreover, two honey samples provided by apiarists (samples C and D) produced inhibition of growth at dilutions of 50%. Sample D showed the highest antibacterial activity. This honey sample was collected from an apiary located in an autochthon forest. Thus, it may be speculated that its higher antibacterial activity might be due to a higher propolis content of the hives.

The results shown by honey samples in relation to *S. aureus* may be important, given that in recent decades there has been a marked increase in difficult-to-treat skin and underlying tissue infections associated with *S. aureus* (Halcón and Milkus, 2004). It has been informed that *S. aureus* has developed resistance against several antibiotics and that it is the principal contaminant agent in many clinical infections (Moreno et al., 2005). Thus, new strategies to treat wounds infected with *S. aureus* are needed, and the possibility to use honey appears as a convenient and less costly treatment option.

Honey also markedly inhibited the growth of *S. epidermidis*. Overall 9 out of the 15 honey samples (60%) inhibited this bacteria's growth. No difference in antibacterial activity was observed between honey samples provided by apiarists and those provided by the honey packers.

The growth of *P. aeruginosa* was inhibited by 3 out of the 15 honey samples (20%) studied in this work. The antibacterial activity of the honey samples provided by apiarists was the same as that of honey samples provided by honey packers. *P. aeruginosa* is usually found in skin wounds, particularly those related to burns. It causes a variety of systemic infections, particularly in victims of severe burns (Yau et al., 2001). Therefore, the antibacterial activity showed by the honey samples studied against *P. aeruginosa* may be of importance in the development of ointments for the treatment of skin wounds.

A noticeable effect of honey provenance on its antibacterial activity against the other bacterial species under study was observed. Honey samples provided by apiarists inhibited the growth of *S. uberis*,

E. coli, and *K. pneumoniae*. These bacterial species were inhibited by 50, 40, and by 20% of the honey samples, respectively. None of the honey samples provided by honey packers showed activity against the aforementioned bacterial species. Although some antibacterial activity could have been expected for all the bacterial species, surprisingly, *M. luteus* and *E. faecalis* bacterial growth were not affected in a noticeable mode by any of the honey samples studied.

The antibacterial activity of honey against *S. uberis*, *E. coli*, and *K. pneumoniae* is of great consequence considering that *Streptococcus* species are recognised pathogens, along with *S. aureus* and coliforms. *S. uberis*, an environmental organism, is the most common pathogen agent isolated from clinical mastitis and the main cause of re-infection during the dry period. It has been informed that *S. uberis* is more resistant to antibiotics than other *Streptococcus* species like *S. dysagalactiae* or *S. agalactiae* (Guérin-Flaubée et al., 2002).

The difference in antibacterial activity of honey samples provided by apiarists, as compared to those provided by honey packers, may be explained by taking into account that honey samples provided by apiarists came from one apiary and it may be reasonable to think that the bees foraging activities mainly concentrated on a few floral species.

The lowest minimum inhibitory concentration of the studied honey samples was 45 g of honey per 100 mL honey solution (sample D). This result is over four times higher than those published by Molan et al. for Manuka honey, a very popular and economically important product derived from the native Manuka tree (*Leptospermum scoparium*), with recognised antibacterial properties (Molan, 1992), they found a CIM value below 10% against *S. aureus* strains (Cooper et al., 1992). The results found in this study are promising, and further research is being conducted on local unifloral honeys seeking for higher antibacterial activities.

The phenol coefficient values of the honey samples studied are smaller than those of ointments regularly used to treat skin infections. However, considering the secondary effects these ointments can cause they are generally used at low concentrations (Jawetz et al., 1990). On then contrary, honey can be regarded as a safe ointment and used without dilution (Molan, 2002).

Acknowledgements

The authors thank Iliana A. Martínez for the language assistance and Secretaría de Ciencia y Técnica of Universidad Nacional de Río Cuarto (SeCyT-UNRC) for the financial support. JMM is a permanent research fellow of Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET).

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